and mixtures of biotinylated and non-biotinylatd peptides are capable of electrostatically neutralizing plasmid DNA and eliminating its electrophoretic mobility.

101. The availability of the biotin groups on the PLL for tethering to a surface was determined using the affinity of biotin for neutravidin (non-glycosylated avidin). Surface-associated DNA was visualized for DNA complexed with K_{150}-B. Fluorescence images taken after the initial incubation on the surface and before the wash demonstrates the presence of complexes across the entire surface (results not shown). Thorough washing of the surfaces resulted in a reduction of the quantity of surface-associated DNA. All subsequent studies used surfaces that were thoroughly washed to ensure binding specificity of the complexes.

102. The quantity of surface associated DNA was subsequently measured as a function of the PLL peptides and the number of biotin groups per complex. Non-biotinylated peptides used for DNA condensation resulted in low surface densities (< 0.1 μg DNA / cm^2) due to non-specific adsorption. The use of biotinylated peptides for DNA complexation increased the surface density of DNA relative to the condition of no biotin groups ($p < 0.05$), suggesting that biotin groups are available on the DNA complexes for interactions with the surface-associated neutravidin. The maximal amount of surface-associated DNA was observed for DNA complexes formed solely with biotinylated peptides ($p < 0.001$), with densities of 2.9 and 4.3 μg DNA / cm^2 obtained for K_{150}-B and K_{19}-B respectively (Figs. 9-10). Complexes formed from either K_{150}-B or K_{19}-B were calculated to have an average number of biotin groups equal to 1705 and 4342 respectively. These surface densities correspond to a tethering efficiency (mass DNA on surface/mass DNA added) of 24% (K_{150}) and 35% (K_{19}). For the K_{150}-B and K_{19}-B, decreasing the amount of DNA incubated on the surface was found to decrease the amount of surface-associated DNA (data not shown). The quantity of surface-associated DNA was also found to decrease as the number of biotin groups in the DNA/PLL complex decreased. The molecular weight of polylysine, both biotinylated and non-biotinylated also affected the quantity of surface-associated DNA. For the biotinylated peptide K_{150}-B, fewer numbers of biotin groups were required to obtain an equivalent amount of surface associated DNA as for the peptide K_{19}-B. However, the use of the non-biotinylated peptide K_{19} for complexation resulted in increased DNA surface densities relative to the use of K_{150}.

103. Culture of HEK293T and NIH/3T3 cells on the DNA modified surfaces led to cellular transfection at 48 hours, with levels of protein production equal to or greater than that obtained by bulk delivery. The transfection experiments (Example 4) used mixtures of K_{150}-B and K_{19} for DNA complexation because this combination had an increased amount of surface associated DNA relative to the other peptide mixtures, particularly when the complexes had few numbers of biotin groups. Quantification of protein expression levels for HEK 293T demonstrated that complexes with 28 and 426 biotin groups produced the maximal transfection (Fig. 11), which was statistically greater ($p < 0.05$) than other conditions with greater numbers of biotin groups. Expression levels obtained for surface-associated complexes with 1705 and 853 biotin groups were not significantly different from the control conditions, which consisted of bulk delivery of DNA complexes formed from either K_{150} or K_{19}. For bulk delivery, the amount of DNA added in complexes with K_{150} or K_{19} corresponded to the surface quantities of DNA for K_{150}-B (1705 biotin groups) and K_{150}-B/K_{19} (28 biotin groups), respectively. These conditions were chosen as the control conditions to represent the limiting cases of surface associated delivery regarding DNA quantities (0.7 μg and 0.16 μg) and PLL composition (K_{150}, K_{19}). For the NIH/3T3 cells (Fig. 12), expression levels obtained by surface associated complexes with 28, 426, and 853 biotin was significantly greater than that obtained with complexes containing 1705 biotin residues ($p < 0.05$). No significant difference between complexes with 28 biotin groups and its bulk control ($p > 0.05$) and 1705 biotin groups and its bulk control ($p > 0.05$) were found. The distribution of transfected cells throughout the cell population also differed between the delivery mechanisms. For bulk delivery, transfected cells were seen throughout the cell population (not shown); however, surface-mediated delivery resulted in cells that were transfected in clusters. Additionally, the location of transfected cells on the surface was consistent with the location of surface-associated DNA seen with the fluorescently-tagged plasmid.

104. The expression levels of protein at 96 hrs by cells cultured on DNA-modified surfaces increased relative to the that observed at 48 hours and, for all biotinylated DNA complexes, was greater than that obtained by bulk delivery. Maximal expression levels by HEK293T cells was obtained for the complexes containing 28 biotin groups and was statistically significant from all other conditions tested ($p < 0.05$) (Fig. 13). The expression

level decreased as the average number of biotin groups on the complex increased ($p < 0.05$). The complexes formed with K_{150}-B (1705 biotin groups) had the lowest transfection level of the surface associated delivery; however, the expression level was significantly greater than the bulk control ($p < 0.01$). The expression levels for the NIH/3T3 cells were less dependent on the number of biotin groups, yet the decreasing expression levels for increasing numbers of biotin groups was again observed (Fig. 14). For all conditions tested with surface associated delivery of DNA complexes tethered to the surface with biotin groups, an increased level of transfection was observed relative to the delivery of non-biotinylated DNA complexes and the bulk delivery of DNA complexes ($p < 0.01$).

EXAMPLES

EXAMPLE 1. Use of Tethered PLL/DNA Complexes

105. Experiment 1. Plasmid DNA encoding either green fluorescent protein (GFP) or luciferase was complexed with modified poly-L-lysine (PLL) at a ratio of 3.1. PLL was modified by PLL reaction with the bifunctional cross-linker sulfosuccinimidyl 6-[3'-(2-pyridyldithio) -propionamido] hexanoate (Sulfo-LC-SPDP, Pierce) prior to DNA complexation at a 1:1 molar ratio. The PLL/DNA complexes were subsequently incubated with glass slides that were modified with (3-mercaptopropyl) -trimethoxysilane (MPTS, Sigma) to create pendant thiol groups. Following coupling of PLL/DNA complexes to the slide, the surfaces were extensively washed and treated with trypsin to degrade the PLL and release the DNA into solution. The surface density of DNA was determined to be 3.9 ± 0.78 $\mu g/cm^2$. Control slides incubated with PLL/DNA complexes without the sulfo-LC-SPDP tether had a surface density of 0.3 ± 0.1 $\mu g/cm^2$ (Fig. 3).

106. Experiment 2. Glass surfaces were prepared with pendant disulfide groups by initially coupling MPTS to create pendant thiol groups. The thiol groups were then reacted with dipyridyldisulfide to form pendant disulfide groups on the surface. Plasmid DNA encoding GFP was mixed for 30 minutes with PLL at a charge ratio of 1:1 (positive to negative). The polylysine chains in the PLL/DNA complexes was covalently coupled to

cysteine (which had a protected amino group) using EDC and Sulfo-NHS. The PLL/DNA complex was subsequently tethered to the glass slide through the thiol group on the attached cysteine. The glass slides were washed to remove non-specifically bound complexes. To verify attachment of the PLL/DNA complexes, the slides were then treated with trypsin to degrade the PLL and release the DNA into solution. This quantity of DNA in solution was measured using the Hoechst 33258 fluorometric dye. The amount of DNA released into solution was equal to 17.8 ± 3.51 μg, which corresponds to a surface density of 3.93 ± 0.78 (Fig. 4). The efficiency of incorporation was determined to be 13.8 ± 2.7%. Control slides in which cysteine was not complexed to DNA released 0.63 ± 0.18 μg, which corresponds to a surface density of 0.28 ± 0.08 $\mu g/cm^2$. The amount of DNA tethered to the slides was consistent with the amounts typically used in transfections. The integrity of the released DNA was subsequently analyzed by gel electrophoresis and found to be intact.

107. NIH3T3 cells were plated into the well of the 96 well dish containing the tethered DNA prepared as described above. Cells were cultured at 37°C. At various times, the cells were examined using a fluorescence microscope for the expression of GFP. Transfected cells were observed within the wells of the dish.

108. Experiment 3. Tethered DNA complexes were attached to 96 well polystyrene microtiter plates with a streptavidin coating as follows: PLL was reacted with the bifunctional cross-linker sulfo-NHS-LC-Biotin (Pierce) prior to DNA complexing at a 1:1 or 10:1 molar ratio (Biotin:PLL), generating biotinylated PLL. To form the complexes, biotinylated PLL was mixed with DNA at a charge ratio of 3:1 and allowed to self-assemble for 30 minutes. The complexes are formed such that the biotin groups are available for specific coupling to a surface through a streptavidin-biotin interaction. These complexes were subsequently incubated (1-2 hours) in streptavidin modified 96-well plates. Following coupling of PLL/DNA complexes to the wells, the surfaces were extensively washed with TNBS buffer and treated with trypsin to degrade the PLL and release the DNA into solution. The number of biotin groups available for coupling controlled the surface density of DNA. Experiments using 200, 100, and 50 moles biotinylated PLL/moles DNA and the 1:1 molar ratio (Biotin:PLL) PLL, yielded the following surface densities: 1.16 ± 0.01 $\mu g/cm^2$, 2.54 ± 0.05 $\mu g/cm^2$, 2.17 ± 0.08 $\mu g/cm^2$ respectively. Similar experiments with the 10:1 molar ratio yielded surface densities of 2.14 ± 0.16 $\mu g/cm^2$, 0.32 ± 0.05 $\mu g/cm^2$, and 0.41 ± 0.06 $\mu g/cm^2$.

Control slides incubated with PLL/DNA complexes without the sulfo-LC-Biotin tether had a surface density of $0.06 \pm 0.02\ \mu g/cm^2$.

Example 2. Synthesis of Biotinylated Poly-Lysine

109. Plasmid DNA encoding for ß-galactosidase (pNGVL1-ß-gal) was purified from bacteria culture using Qiagen (Santa Clara, CA) reagents and stored in Tris-EDTA buffer solution (10 mM Tris, 1mM EDTA, pH = 7.4). Fluorescein tagged β-galactosidase vector (Fl-ß-gal) was purchased from Gene Therapy Systems (San Diego, CA). Two polylysine (PLL) peptides were used for DNA complexation: Cys-Trp-Lys_{19} (K_{19}, BioPeptide, San Diego, CA) and Lys_{150} (K_{150}, average molecular weight of 20,000, Sigma, St Louis, MO). Avidin and biotin reagents for peptide modification and surface tethering were purchased from Pierce (Rockford, IL). All other reagents were obtained from Fisher Scientific (Fairlawn, NJ) unless otherwise noted.

110. Peptide K_{19} was modified with a biotin group through the terminal cysteine residue by reaction of the sulfhydryl group with the iodoacetyl group of the biotinylation reagent, EZ-link-PEO-Iodoacetyl-Biotin. K_{19} (10 mg) was dissolved in 850 µL of buffer (50 mM Tris, 5 mM EDTA, pH = 8.3) that was previously bubbled with nitrogen gas. The EZ-link-PEO-Iodoacetyl-Biotin (3.8 mg) was also dissolved in 150 µL of buffer (0.1 M Sodium Phosphate, 5 mM EDTA, pH = 6.0). The biotin solution was added dropwise to the peptide solution, mixed gently, covered with aluminum foil and incubated for 90 minutes. The starting peptide solution and the reaction mixture were analyzed by HPLC to determine if the reaction had gone to completion. The starting peptide solution and the reaction mixture were resolved by injecting 20 µg through a C18 RP-HPLC column eluted with water (0.1% trifluoroacetic acid (TFA)) and a acetonitrile gradient (0.1% TFA, 0 to 95% over 50 minutes at 60°C) while detecting the absorbance at 260 nm. For purification, sephadex (G15) was equilibrated in deionized water for 30 minutes prior to packing in a glass column (2 cm diameter x 12 cm height). The reaction mixture was passed though the column using deionized water. Twenty fractions were collected and the presence of the tryptophan side chain was examined by measuring the absorbance at 260 nm (Beckman Instruments Inc., Fullerton, CA). The fractions with the greatest absorbance at 260 nm were lyophilized

(Labconco Corp., Kansas City, MO) and analyzed by mass spectrometry. The purified biotinylated peptide (K_{19}-B) was stored as a powder at –20 °C.

111. Peptide K_{150} was biotinylated using succinimide ester (NHS)/amine chemistry. K_{150} (10 mg) was dissolved in 1 mL of phosphate buffered saline (PBS, pH = 7) and EZ-link-Sulfo-NHS-LC-Biotin (2.8 mg) was added directly to the solution, mixed gently and incubated for 2 hours at 4°C. The reaction mixture was purified using dialysis cassettes immersed in deionized water. The dialyzed product was further purified using a monomeric avidin column to separate the biotinylated components from non-biotinylated species. The biotinylated product was eluted with 10 ml of a 10 mM biotin solution and dialyzed to remove the unconjugated biotin. The purified biotinylated peptide (K_{150}-B) was then lyophilized and stored as a powder at –20 °C. The degree of biotinylation of K_{150}-B was determined by quantifying the mole ratio of biotin to K_{150} using 2[4'-hydroxyazobenzene]-benzoic acid (HABA). The absorbance at 500 nm of a HABA/ avidin solution in PBS was recorded before and after the addition of the K_{150}-B and used to calculate the molar ratio of biotin to K_{150}.

Example 3. Complex formation and surface tethering.

113. The ability of the biotinylated and non-biotinylated polylysine (K_{150}, K_{150}-B, K_{19}, K_{19}-B) synthesized as described above, to condense DNA was assessed by gel electrophoresis. Biotinylated and non-biotinylated peptides were mixed and added in a stepwise manner (1 µL of 1 mg/mL) to a DNA solution (200 µL of 20 µg/mL). After each addition step, the solution was vortexed for 4 seconds, incubated for 10 min and a sample (10 µL) removed. Upon complete addition of peptide, trypsin was added to digest the polylysine. Gel electrophoresis was performed to assess the extent of complex formation for the samples and the trypsin-digested DNA solution.

114. DNA/PLL complexes were incubated on surfaces to specifically tether the complexes through the biotin-neutravidin binding. DNA (90 µL of 44.4 µg/mL) was complexed at a charge ratio (+/-) of 5.5:1 with the four peptides (K_{150}, K_{150}-B, K_{19}, or K_{19}-B) individually or with mixtures of biotinylated and non-biotinylated peptides. The number of tethers on each complex is varied by mixing biotinylated and non-biotinylated PLLs prior to complexation with DNA. Complexes were incubated after mixing for 30 min at room